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Detection, immunoabsorption, and inhibition of cytotoxic activity of anti-alpha-Gal antibodies using newly developed substances with synthetic Gal alpha-1-3Gal disaccharide epitopes

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The presence of naturally occurring anti-Gal-alpha-1-3Gal (anti-alpha-Gal) antibodies in human serum is believed to be a major factor in the hyperacute rejection of discordant organ xenografts such as the pig-to-human combination. Gal-alpha-1-3Gal epitopes are expressed on pig tissues and the binding of anti-alpha-Gal leads to endothelial cell activation and complement-mediated, hyperacute graft rejection. One possible method to overcome this problem is to absorb anti-alpha-Gal antibodies from the plasma of the xenograft recipient using a suitable immunoabsorbent or to interfere with their binding to tissues and thus

prevent their cytotoxic activity by the intravenous injection of soluble antigen. We describe here the use of new synthetic antigens containing the Gal-alpha-1-3Gal disaccharide (Bdi) epitope. Soluble conjugates of the Bdi with polyacrylamide (PAA-Bdi) were used as coating antigens for an anti-alpha-Gal ELISA as well as for in vitro inhibition of the cytotoxicity of anti-alpha-Gal. An immunoabsorbent consisting of PAA-Bdi coupled to macroporous glass (Sorbent Bdi) was tested for absorption of anti-alpha-Gal from human serum. Anti-alpha-Gal IgM, IgG and IgA could be detected by the anti-alpha-Gal ELISA and were specifically absorbed by Sorbent Bdi with absorption efficiencies ranging from 70 to 50% for anti-alpha-Gal IgG and 60 to 25% for anti-alpha-Gal IgM. A comparison of the anti-alpha-Gal absorption by Sorbent Bdi and rabbit red blood cells revealed a qualitatively (isotype distribution) and quantitatively similar pattern. Nonspecific absorption by Sorbent Bdi was low, as detected by the reduction of anti-A trisaccharide antibodies. The cytotoxic effect of human serum on pig kidney (PK15) cells was almost totally inhibited by the addition of synthetic B disaccharide or by adsorption of the serum through immunoaffinity columns of PAA-Bdi. We conclude that the newly developed, synthetic alpha-Gal-1-3Gal antigens are suitable for the detection and immunoabsorption or inhibition of anti-alpha-Gal antibodies.

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